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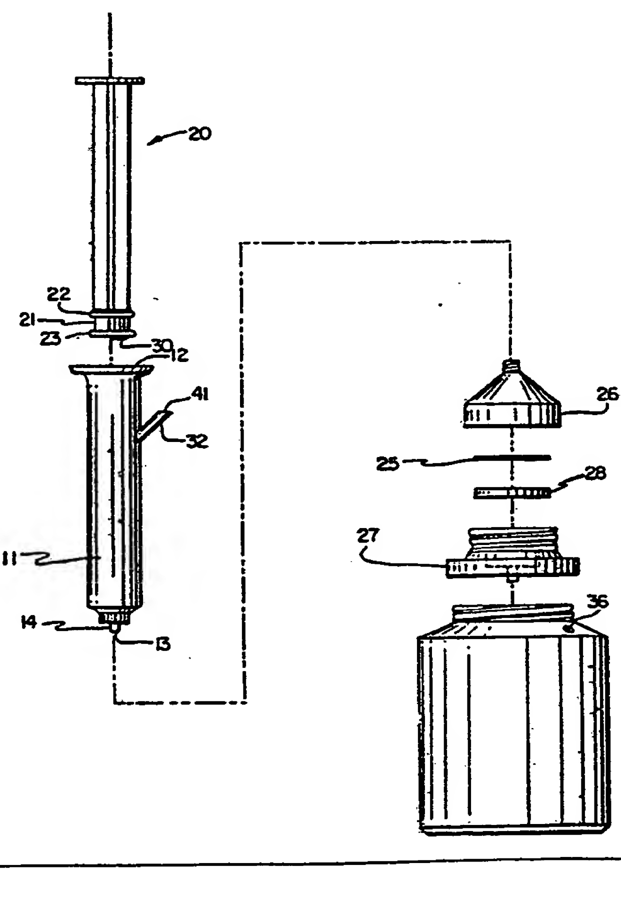
(54) Title: PRESSURE-ASSISTED ANALYTICAL APPARATUS AND METHOD

(57) Abstract

An apparatus and methods for assaying a test substance utilizing filter means (25) and superatmospheric pressure. The apparatus has a reaction chamber (11) with an open end (13), a porous filter (25) communicating with the open end (13), the filter (25) having pores sized to prevent passage therethrough of a complex of labeled antibody and analyte, the reaction chamber (11) being cylindrical and having a plunger (20) snugly received therein for providing superatmospheric pressure to the contents of the reaction chamber (11) during use.



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PRESSURE-ASSISTED ANALYTICAL APPARATUS AND METHOD

BACKGROUND OF THE INVENTION

The present invention relates to an apparatus and method for determining the presence of microorganisms, chemicals, and other analytes in physiological, biological and environmental specimens.

The traditional methods of detecting and identifying microorganisms in physiological and biological fluids require cell culturing on laboratory media (sometimes followed by susceptibility testing to determine resistance to a particular antibiotic), identification of the organism by detection of serum antibodies against the organism, or isolation of the organism from infected tissues. These methods may require from 24 hours to ten days to perform and therefore do not necessarily contribute toward patient management and diagnosis. In some cases the microorganism cannot be isolated or cultured. Development of rapid detection methods which are sensitive enough to determine if a particular microorganism (viable or non-viable) is present in a biological specimen and which can be easily and rapidly performed in a clinic or doctor's office would greatly aid in the diagnosis of illnesses induced by such organisms.

Membranes and filters have been used in a number of devices and in procedures for determining the presence of an analyte in a sample. These devices,

however, are dependent on a specific sample size, typically utilize bound reagents and are dependent upon the rate of diffusion of liquid sample through an absorbent material to define the time limitations of the assay. For example, U.S. Patent 4,632,901 discloses an apparatus having a membrane or filter to which an antibody is bound and which is in liquid contact with an absorbent material. The sample size is limited by the absorbancy of the material. U.S. Patent 4,623,461 also discloses a membrane device having an absorbent material in fluid contact with the membrane. In this device, however, the absorbent material contacts only the periphery of the membrane thus causing liquid to diffuse transversely. The device is useful for only a limited number of sample types.

SUMMARY OF THE INVENTION

The present invention provides apparatus and methods for use in determining, in a liquid sample, the presence of an analyte which is one member of a ligand-receptor pair; the method using filter means and superatmospheric pressure. We have found that the use of superatmospheric pressure with the apparatus and methods of the invention to create a pressure differential across the filter means provides substantially better results than are obtained with use of a vacuum to create the pressure differential.

The apparatus of the invention comprises a reaction chamber having at least one open end, a predetermined quantity of a labeled member of the ligand-receptor pair of which the analyte is the other member, the labeled ligand-receptor pair member being chosen to bind to the analyte in relation to the quantity of analyte in the sample and being disposed within the chamber, means for applying

superatmospheric pressure to the contents of the reaction chamber, and filter means communicating with the open end of the reaction chamber for preventing passage through the filter means of a labeled ligand-receptor pair member when it is bound to an analyte while permitting unbound labeled ligand-receptor pair member to pass therethrough when superatmospheric pressure is applied to the chamber to force the contents of the chamber through the filter means.

In one embodiment of the invention, the filter means comprises a porous filter or membrane communicating with the open end of the reaction chamber, the filter having pores sized to prevent passage therethrough of the labeled ligand-receptor pair member when it is bound to analyte while permitting unbound labeled ligand-receptor pair member to pass therethrough when superatmospheric pressure is applied to the reaction chamber. The filter is desirably made of a low protein binding material having pores sized no larger than about 100um in diameter and preferably in the range of .45um-15um.

In another embodiment of the invention, the apparatus provides a substantially enclosed system comprising a cylindrical chamber having a plunger received snugly therein for providing superatmospheric pressure to the contents of the reaction chamber when the assay is performed, a filter, and support means for supporting the filter between an upper and lower housing, the upper housing being made of a clear material so that the surface of the filter is visible through the housing wall. The upper and lower housings are removably attachable to the reaction chamber and to a collection chamber that is positioned to collect fluid and other material that passes

through the filter during the performance of an assay.

In another embodiment, the invention comprises a method of determining, in a liquid sample, the presence of an analyte which is one member of a ligand-receptor pair. The method comprises the steps of reacting the liquid sample with a predetermined quantity of a labeled member of the ligand-receptor pair chosen to bind to the analyte in the sample in relation to the quantity of analyte present in the sample, forcing the resulting reaction mixture under superatmospheric through a porous filter having pores sized to substantially prevent passage therethrough of the labeled ligand-receptor pair member when it is bound to an analyte while permitting unbound labeled ligand-receptor pair member to pass therethrough, and determining the presence of analyte in the sample by detecting the presence of labeled ligand-receptor pair member in and on the filter.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an exploded front view of an apparatus of the invention;

Figure 2 is a cross-sectional view of the apparatus of Figure 1; and

Figure 3 is a broken-away, front view of a plunger part of the apparatus of Figure 1.

DESCRIPTION OF PREFERRED EMBODIMENTS

As used herein, "ligand-receptor pair" refers to a pair of compounds of which one, a "receptor" is capable of recognizing a particular spacial and polar organization of the other ("ligand") or portion thereof, and is capable of binding to that compound. For various ligands, illustrative receptors forming the other half of a ligand-receptor pair include antibodies, enzymes, lectins, antibody fragments, complementary nucleic acids, protein A, anti-idiotype

antibodies, avidin, and the like. Commonly, the receptor will be an antibody and the ligand will act as an antigen or hapten. Desirably, the analyte or an analyte derivative will be the ligand. As used herein, "analyte derivative" means a chemical derivative of an analyte that retains the capacity to bind to the other member of a ligand-receptor pair as does the analyte.

By "labeled member of a ligand-receptor pair" is meant a conjugate of a ligand-receptor pair member with a chemical label such as an enzyme, a fluorescent compound or other detectable chemical species, the conjugate retaining the capacity to bind to the other member of the ligand-receptor pair, and the enzyme or other detectable label continuing to have a capacity of being detected by a detector system (which may be a separate chemical reaction system) to provide a perceptible signal. "Detector," "label detector," "detection system," and the like, as exemplified below, refers to a chemical system that provides perceptible signals, commonly electromagnetic radiation or absorption of the same leading to perceptible fluoresense, color changes and the like, when contacted with a specific enzyme or other label and desirably with substrate and chromogen.

Several convenient, known chromogens are available which produce visible color when added to their specific enzymes and substrates. For example, o-phenylenediamine and o-dianisidine have been used as peroxidase and glucose oxidase chromogens. Napthol-MX-phosphate and the chromogen Fast Red TR salt are used for detecting alkaline phosphatase. Yolken, R.N., "Solid-Phase Enzyme Immunoassays for the Detection of Microbial Antigens in Body Fluids," in Manual of Clinical Microbiology, pgs. 949-957 (1985)

contains a more detailed description of these chromogens.

The invention is useful in detecting a broad range of analytes. U.S. Patents 4,374,925 and 3,817,837 set out excellent lists of analytes which are part of specific binding pairs, the teachings of which patents are incorporated herein by reference. Analytes of particular interest include viruses, bacteria, fungi and other analytes of similar size. Examples of analytes that can be assayed with this invention include Chlamydia, Salmonella, Bordetella, Candida and Aspergillus.

A preferred embodiment of the apparatus of the invention is shown as 10. A reaction chamber, typified as a transparent hollow column of plastic or the like 11 has an upper opening 12 and a lower opening 13. The reaction chamber may be sized and shaped to hold any volume of sample sufficient for performing an immunoassay; preferably, the chamber will hold approximately 3 ml of sample. It may be made of glass or any suitable plastic material. As best shown in Figure 1 the sidewalls of the chamber at its lower end desirably slope inwardly to form a constriction 14. The sloping structure of the reaction chamber walls provide an integral funnel to facilitate the exposure of liquid sample and wash solutions to the walls of the vessel when an assay is performed.

Disposed within the chamber is a labeled ligand-receptor pair member chosen to bind to the analyte in relation to the amount of analyte in a liquid sample. The labeled ligand-receptor pair member may be dried onto the inner wall of the reaction chamber, placed in the chamber in a dry pellet or as a powder or dissolved in a liquid in the

chamber. Preferably, the labeled ligand receptor pair member is in a pellet form and may be mixed with other inert ingredients, such as stabilizers and solubilizers.

The apparatus also has filter means for preventing the passage therethrough of labeled ligand receptor pair member bound to analyte while permitting unbound labeled pair member to pass therethrough when superatmospheric pressure is applied to the reaction chamber. The filter means may be any of a variety of membranes or filters that separate materials by size, charge and the like. Preferably, the filter means is a porous filter or membrane having pores sized to prevent the passage therethrough of the labeled pair member when it is bound to the analyte while permitting unbound labeled pair member to pass therethrough when superatmospheric pressure is applied to it.

The filter is desirably made of a low protein binding material, such as derivatized nylon, polysulfone and the like. For use in this invention, a low protein binding material binds less than about 0.1% of protein that passes through it under the following conditions. 0.4 mls of phosphate buffered saline ("PBS") with 3% polyethylene glycol and 100 ul (48ug) of tritiated human serum albumin ("HSA") are forced through the filter material having a diameter of about 13mm of which about 10mm contacts the protein solution and a thickness of about .15mm (volume 10.8mm^3) being tested using the apparatus of the invention. The filter is then washed by forcing 5 mls of a wash solution (PBS with 0.05%, polyoxyethylene sorbitan monolaurate (Tween 20, a trademarked product of Sigma Chemical)) through it. The filter is removed and placed in 4 mls of scintillation cocktail. The

amount of tritiated HSA bound to the filter is calculated using disintegrations per minute measured over five minutes. Using this procedure, cellulose acetate, polysulfone and derivatized nylon 66 filters bound less than 0.1% of the HSA. The filter material is also desirably deformable or compressible in the direction of fluid flow.

The pore size of the filter used in this invention will vary according to the analyte being assayed for and the type of sample. The pores should be sized according to the particular analyte for which the assay is being performed so that when the analyte is bound to the labeled ligand receptor pair member the resulting complex is prevented from passing through the filter. Although the pore size of the filter may be larger than the average diameter of the organism being assayed in order to obtain adequate sensitivity of the assay the pore size is desirably no more than 2.5 times as large as the analyte being assayed. Filters with pore sizes ranging from 0.1um in diameter to 100um in diameter have been used with this invention. Preferably, filters having pores sized no larger than about 15um are used.

Other factors may influence the selection of pore size of the filter besides analyte size. For example, the type of specimen being assayed may contain biological agents and/or debris which may clog the filter pores and thus interfere with the assay. It is desirable to select a pore size that will allow such agents and/or debris to pass through the filter when superatmospheric pressure is applied but still prevents the passage of bound labeled ligand receptor pair member therethrough. Similarly, the size of the labeled ligand receptor pair member selected to bind the analyte is also important. The pores must be

large enough to allow unbound labeled ligand receptor pair member to pass through the filter when superatmospheric pressure is applied.

As shown in Figure 2 the porous filter or membrane 25 is placed between upper and lower housings 26 and 27 on a filter support 28. The filter communicates with the reaction chamber through constriction 14. The upper housing 26 desirably forms a compartment 29 above the filter 25 and is sealingly engaged to the outer sidewalls of the reaction chamber. In the preferred embodiment, the constriction 14 of the reaction chamber is sized and shaped so that if the filter is wetted to make it substantially airtight when liquid is added to the reaction chamber, an "airlock" will form that will prevent passage of the reaction mixture into compartment 29 until superatmospheric pressure is applied. When this "airlock" is positioned directly above the filter surface during the assay, nonspecific adsorption of the labeled ligand receptor-pair member and potentially interfering substances is significantly minimized by decreasing the length of time those agents contact the filter surface.

The walls of the upper housing desirably slope outwardly as shown in Figures 1 and 2 to provide even dispersion of solutions over the filter surface during performance of the assay. Similarly the walls of the lower housing slope inwardly to provide a funnel for directing the flow of materials passing through the filter. The upper housing is desirably made of a clear material so that color change occuring on the filter surface can be observed by the user without the necessity of removing the filter. Preferably, the upper and lower housings are removably attached so that the filter may be removed from the apparatus

after the assay has been performed and retained as a record of the assay results.

The apparatus of the invention further includes means for applying superatmospheric pressure to the reaction mixture in the reaction chamber. The pressure means may be any of a variety of conventional means but preferably comprises a plunger slidingly received within the upper opening 12 of the reaction chamber 11. Other means that may be used with this invention include a bellows that sealingly connected with the reaction chamber or a source of compressed gas which when opened, releases a specific amount of gas at a specific rate to apply pressure to the reaction mixture disposed in the reaction chamber. The means should be able to provide at least 1-5 psi (gauge pressure) to the contents of the reaction chamber so that liquid passes through the filter at a rate of about 1 milliliter in 12-25 seconds.

The plunger comprises a piston 21 having sealing rings 22, 23 that sealingly engage the inner sidewalls of the reaction chamber when received therein and a handle for sliding the piston upwardly and downwardly within the chamber. The piston desirably includes one-way valve means for allowing air to escape from the reaction chamber when the plunger is moved upwardly to prevent disturbance of reactants lying on the filter surface.

In Figure 3, the valve means is typified as a flexible flap 30 normally covering an axially extending hole 31 formed in the piston 21, so that when the the piston is moved upwardly the flap 30 opens allowing passage of air through the piston hole and when the piston is moved downwardly the flap remains in its normal position covering the hole in the piston.

In the preferred embodiment, the apparatus of the invention is substantially enclosed to protect the user from exposure to undesirable elements in the liquid sample. A collection chamber 35 is positioned below the lower filter support in liquid communication with the reaction chamber to collect liquid and unbound ligand-receptor pair member that passes through the filter when superatmospheric pressure is applied to the reaction chamber. The collection chamber may be of any variety of size and shape and may be attached to the reaction chamber or filter housings by any of a variety of means. It may be integrally formed with the reaction chamber having a filter disposed therebetween, or it may be attached to the apparatus using any of a number of conventional attachment means such as complimentary screw threads, adhesive and the like. As shown in Figure 1, in the preferred embodiment the collection chamber is removably attached to the lower filter housing with complimentary screw threads.

The collection chamber desirably includes means for releasing gas from within the chamber and desirably comprises a hole 36 formed in the wall of the collection chamber as shown in Figure 1. The gas releasing means may further comprise filter means which substantially prevents the release of particles or liquid from the collection chamber. The filter means may be an absorbent material placed within the collection chamber that absorbs liquid as it passes through the filter or it may simply be porous filter attached to the wall of the collection chamber covering the gas releasing means.

In another embodiment of the invention, the upper opening of the r action chamber is closed by the plunger, the removal of which is prevented by

retaining means, such as protrusion arms 40 or an inwardly projecting flange (not shown) formed at the top end of the chamber. This apparatus comprises means for allowing the introduction of liquid sample into the reaction chamber. Sample introducing means is typified in Figures 1 and 2 as a tubular port 32, although it may be any variety of conventional means, such as a hole formed in the wall of the reaction chamber. Preferably,the sample introducing means is designed to prevent escape of liquid sample out of the reaction chamber. This may be accomplished by covering the opening into the reaction chamber with a diaphram 41 that may be penetrated by a needle and which will then reseal itself after removal of the needle or it may be a valve which may be selectively opened or closed, such as a stopcock.

The opening of the sample introducing means into the reaction chamber should be positioned so that a cushion of air can be trapped in the reaction chamber between the reaction mixture and the means for applying superatmospheric pressure. The cushion of air is useful in pushing all liquid through the filter to ensure that traces of unbound labeled ligand receptor pair member do not remain trapped in liquid on the filter surface.

The method of this invention comprises reacting liquid sample suspected of containing analyte, the analyte being a member of a ligand-receptor pair, with a predetermined quantity of a labeled member of the ligand-receptor pair chosen to bind to the analyte in the sample in relation to the quantity of analyte present in the sample, forming a reaction mixture. Preferably, liquid sample is added to the reaction chamber of the apparatus described above in which a predetermined quantity of the labeled member of the

ligand-receptor pair is present. The reaction mixture remains in the reaction chamber a predetermined length of time to allow the labeled pair member to bind to analyte in the sample. This "incubation" time is typically 5-10 minutes. The resulting reaction mixture is then forced under pressure through a filter having pores sized to substantially prevent passage therethrough of the labeled ligand-receptor pair member when it is bound to analyte while permitting unbound labeled pair member to pass therethrough. Typically, a wash solution such as PBS or PBS/ 0.05% Tween 20 is added to the reaction chamber and superatmospheric pressure applied to force the solution through the filter to wash through any remaining unbound labeled ligand-receptor pair member.

The presence of analyte in the sample is detected by detecting the presence of labeled ligand-receptor pair member in and on the filter. The presence of analyte may be determined using any of a number of detection means. Preferably, however, after the reaction mixture has been forced through the filter by applying superatmospheric pressure a liquid detection system responsive to the label in the ligand-receptor pair member is forced through the filter. If analyte is present, color appears on the filter surface. The filter may be removed and the filter checked for the appearance of color at predetermined intervals or it may be left in the apparatus and viewed through the clear wall of the upper housing.

Using the method and apparatus of this invention we are able to obtain rapid, reproducible assay results that are superior to results obtained using a vacuum to draw liquid sample through the filter means. The use of superatmospheric pressure appears

to result in the trapping of the complexes of analyte bound to labeled ligand-receptor pair member upon the filter surface, whereas this trapping appears to occur primarily within the body of the filter matrix when a vacuum is used. It is desirable to have the complexes trapped on the filter surface so that label on the complexes can fully react with the substrate/chromogen or other detection system and so the resulting color change can be easily detected visually.

Although we do not wish to be bound by the following explanation, we theorize that the use of superatmospheric pressure with the porous filters used in this invention cause these filters to slightly deform or compress so that the analyte-labeled ligand receptor pair member complexes do not readily enter the filter pores. That is, the compression or deformation of the filter when superatmospheric pressure is applied to a surface of the filter may cause the pores at that surface to become changed in size so that the filter more readily collects the complexes on that surface.

The invention may be better understood by reference to the following non-limiting examples.

<u>Example 1- Detection of Chlamydia</u>

The labeled ligand receptor pair member used is horseradish peroxidase ("HRP") labeled polyclonal antibody to <u>Chlamydia</u>. Although the antibody, conjugate preparation used comprises a polyclonal antibody, monoclonal or monospecific antibodies or combinations may also be used. Techniques for polyclonal, monoclonal, or monospecific antibody preparation are well known.

The conjugate was prepared as follows. Periodate activation of 4 mgs HRP involved the oxidation of the carbohydrate moiety with 0.2 ml of

0.1 M sodium m ta-periodate to form free aldehyde groups. The solution was stirred for 20 minutes at room temperature. Following dialysis with 1mM sodium acetate buffer, pH 4.4 at 4°C, the purified antibody (8mgs of IgG) was added to the peroxidase solution at a slightly alkaline pH (pH 9.5) followed by reduction of the resulting Schiff base by addition of 0.1 ml of 4 mg/ml sodium cyano borohydride to stabilize covalent bonds between the primary amino groups on the antibody and aldehyde groups of the peroxidase (stirred at 4°C for 2 hours).

Following the coupling reaction, the conjugate preparation was purified on a Sephacryl S-200 gel permeation chromatography column to separate uncoupled proteins from conjugated proteins.

The assay was conducted using a derivatized nylon 66 filter (LoProdyne, a trademarked product of Pall) having .45um sized pores. The filter was wetted and then inserted into the apparatus shown in Figure 1 by unscrewing the upper housing from the lower housing and placing the filter on the filter support carried by the lower housing. The filter housings were screwed together and attached to the reaction chamber and collection chambers. The plunger was placed in the upper opening of the reaction chamber and pushed down until the piston rested just above the opening of the sample introducing arm.

Four units were prepared, two for a positive test and two for controls. Each positive unit received 300 ul of PBS and each control unit received 400ul of PBS through the sample introducing arm. 100ul of a 1:100 stock culture dilution of Chlamydia was added to the reaction chamber of each positive unit through the sample introducing arm. 100 ul of 9mgs/ml HRP-anti-Chlamydia was already present in the

reaction chamber of each unit. The reaction mixture of each unit was incubated in the chamber for approximately ten minutes. The plunger in each unit was then pressed downwardly with about 1-5 psi (gauge pressure) to cause the reaction mixture to pass through the filter at a rate of about 1ml per 12 to 25 seconds.

The plunger in each unit was then pulled back so that the piston was positioned above the opening of the sample introducing arm and 5 ml of PBS/Tween 20 wash solution was added to the chamber. The plunger was pushed down to force the wash solution through the filter and the plunger returned to its original position. 100ul of substrate/chromogen (0.5 mg/ml 4-chloro-1-naphthol plus 0.0006% H_2O_2 was then added to the reaction chamber and forced through the filter. Analyte present in the sample caused labeled antibody present on the surface to interact with substrate/chromogen and turn grey or deep purple so that the filter appears to become grey or deep purple if analyte was present. Each filter was checked at 30 seconds, 1 minute, 2 minutes, 5 minutes and 10 minutes to determine whether a color appeared.

A positive test is recorded as a plus 1 or greater using a color chart (such as a Pantone chart selector #534 produced by Pantone, Inc.); or a reading
27 of 10 or above using a reflectance spectrophotometer. When the reflectance spectrophotometer was used the colorimetric data were calculated from the spectral data measured by the sensor. The total color difference (DE) was measured by the L, a, b coordinate system, and was recorded in units. The total color difference value depends on the standard illuminant and observer used. The total color difference data obtained is a composite unit and depends upon the a)

dark/light difference; b) red/green difference; c) yellow/blue difference; and d) chromaticity difference (chromaticity and hue). The higher the value of DE, the more saturated and chromatic was the color.

The total assay time was about 25 minutes including the time for final readout at 10 minutes. The units to which Chlamydia was added gave a positive colored signal while the negative controls were white or negative even after 10 minutes.

Example 2- Detection of Candida

In this example the labeled ligand receptor-pair member was a 1:75 dilution of 11.08mgs/ml HRP-labeled polyclonal antibody to Candida. The conjugate was prepared according to the procedure described in Example 1. The same type of filter was used and the apparatus assembled as described in Example 1.

In this example Candida organisms (10^5 - 10^7 organisms) were added to urine and added to a buffer solution. Urine without Candida and a buffer solution without Candida were used as negative controls. The assay was carried out as previously described except the wash solution used was PBS not PBS/Tween.

All urine or buffer samples containing Candida showed a positive reaction. The negative controls showed no color after 5 minutes. The total assay time was twenty minutes including color development time.

WHAT IS CLAIMED IS:

1. An apparatus for use in determining in a liquid sample the presence of an analyte which is one member of a ligand-receptor pair comprising:

a reaction chamber having at least one open end;

a predetermined quantity of a labeled member of the ligand-receptor pair chosen to bind to analyte in the liquid sample in relation to the quantity of analyte in the sample within the reaction chamber;

means for applying superatmospheric pressure to the reaction chamber to force the contents therein through the open end of the reaction chamber; and

filter means communicating with the open end of the reaction chamber to prevent passage therethrough of the labeled pair member when it is bound to analyte while permitting unbound labeled pair member to pass therethrough when superatmospheric pressure is applied to the reaction chamber.

2. An apparatus for use in determining in a liquid sample the presence of an analyte which is one member of a ligand-receptor pair comprising:

a reaction chamber having at least one open end;

a predetermined quantity of a labeled member of the ligand-receptor pair chosen to bind to analyte in the liquid sample in relation to the quantity of analyte in the sample disposed in the reaction chamber;

means for applying superatmospheric pressure to the reaction chamber; and

a porous filter communicating with the open end of the reaction chamber, the filter having pores sized to prevent passage therethrough of the labeled pair member when it is bound to analyte while permitting unbound labeled pair member to pass therethrough when superatmospheric pressure is applied to the reaction chamber to force its contents through the filter.

3. The apparatus of Claim 2 wherein the diameter of the filter pores is no larger than about 100um.

4. The apparatus of Claim 3 wherein the diameter of the filter pores is in the range of about .5um-15um.

5. The apparatus of Claim 2 further comprising a collection chamber positioned adjacent the filter to collect liquid that passes therethrough.

6. The apparatus of Claim 5 wherein the means for applying superatmospheric pressure is a piston that fits guidingly and slidably within the reaction chamber.

7. The apparatus of Claim 5 wherein the collection and reaction chambers are sealingly engaged and the filter is disposed therebetween.

8. The apparatus of Claim 7 wherein the collection chamber has means for releasing gas from within the chamber.

9. The apparatus of Claim 8 wherein the gas releasing means is a hole formed in a wall of the collection chamber.

10. The apparatus of Claim 9 wherein the gas releasing means further comprises filter means to substantially prevent the release of particles or liquid from the collection chamber.

11. The apparatus of Claim 2 wherein the reaction chamber comprises a tubular member substantially sealingly receiving the piston therein.

12. The apparatus of Claim 11 wherein the reaction chamber further comprises means for allowing the introduction of liquid sample into the reaction chamber.

13. The apparatus of Claim 12 wherein the sample introducing means is a port.

14. The apparatus of Claim 12 wherein the port includes a closure which may be selectively opened or closed.

15. The apparatus of Claim 2 further comprising upper and lower filter housings and support means positioned between the housings to support the filter.

16. The apparatus of Claim 15 wherein the reaction chamber further comprises a constriction at its open end that is spaced from the filter but that communicates with the filter through the upper housing to form a compartment, the constriction being sized and shaped to enable an airlock to be formed in the compartment when the filter has been wetted to make it substantially airtight.

17. The apparatus of Claim 2 wherein the porous filter is a low protein binding material

18. The apparatus of Claim 2 wherein the porous filter is compressible in the direction of fluid flow.

19. A method of determining in liquid sample the presence of an analyte which is one member of a ligand-receptor pair comprising:

reacting liquid sample suspected of containing analyte with a predetermined quantity of a labeled member of the ligand-receptor pair chosen to bind to analyte in the sample in relation to the quantity of analyte present in the sample to form a reaction mixture;

forcing the resulting reaction mixture under pressure through a filter having pores sized to substantially prevent passage therethrough of the labeled pair member when it is bound to analyte while permitting unbound labeled pair member to pass therethrough; and

determining the presence of analyte in the sample by detecting the presence of labeled

ligand-receptor pair member in and on the filter.

20. The method of Claim 19 wherein a solution containing a detection system responsive to the label of the labeled pair member to produce a color is forced under pressure through the filter after the reaction mixture and the presence of analyte determined by observing the appearance of color in and on the filter.

21. The method of Claim 19 wherein unbound labeled pair member is washed through the filter after the reaction mixture is forced through the filter.

22. The method of Claim 19 wherein the labeled ligand receptor pair member is labeled antibody to the analyte.

23. The method of Claim 19 wherein the labeled ligand receptor pair member is labeled with an enzyme.

24. The method of Claim 20 wherein the detection system comprises enzyme substrate and chromogen.

25. A method of determining in liquid sample the presence of an analyte which is one member of a ligand-receptor pair employing a device comprising a reaction chamber having at least one open end; a predetermined quantity of a labeled member of the ligand-receptor pair chosen to bind to analyte in the liquid sample in relation to the quantity of analyte in the sample; means for applying superatmospheric pressure to the reaction chamber; and a porous filter occluding the open end of the reaction chamber, the filter having pores sized to substantially prevent passage therethrough of the labeled pair member when it is bound to analyte while permitting unbound labeled pair member to pass therethrough when superatmospheric pressure is applied to the reaction chamber;

the method comprising:

reacting liquid sample suspected of containing analyte with the labeled member of the ligand-receptor pair in the reaction chamber to form a reaction mixture;

forcing the resulting reaction mixture under pressure through the filter, using the pressure means; and

determining the presence of analyte in the sample by detecting the presence of labeled ligand-receptor pair member in the reaction chamber and on the filter.

26. The method of Claim 19 wherein the labeled ligand receptor pair member is one of the group consisting of antibody, antibody fragments, protein A, anti-idiotype antibody, and avidin.

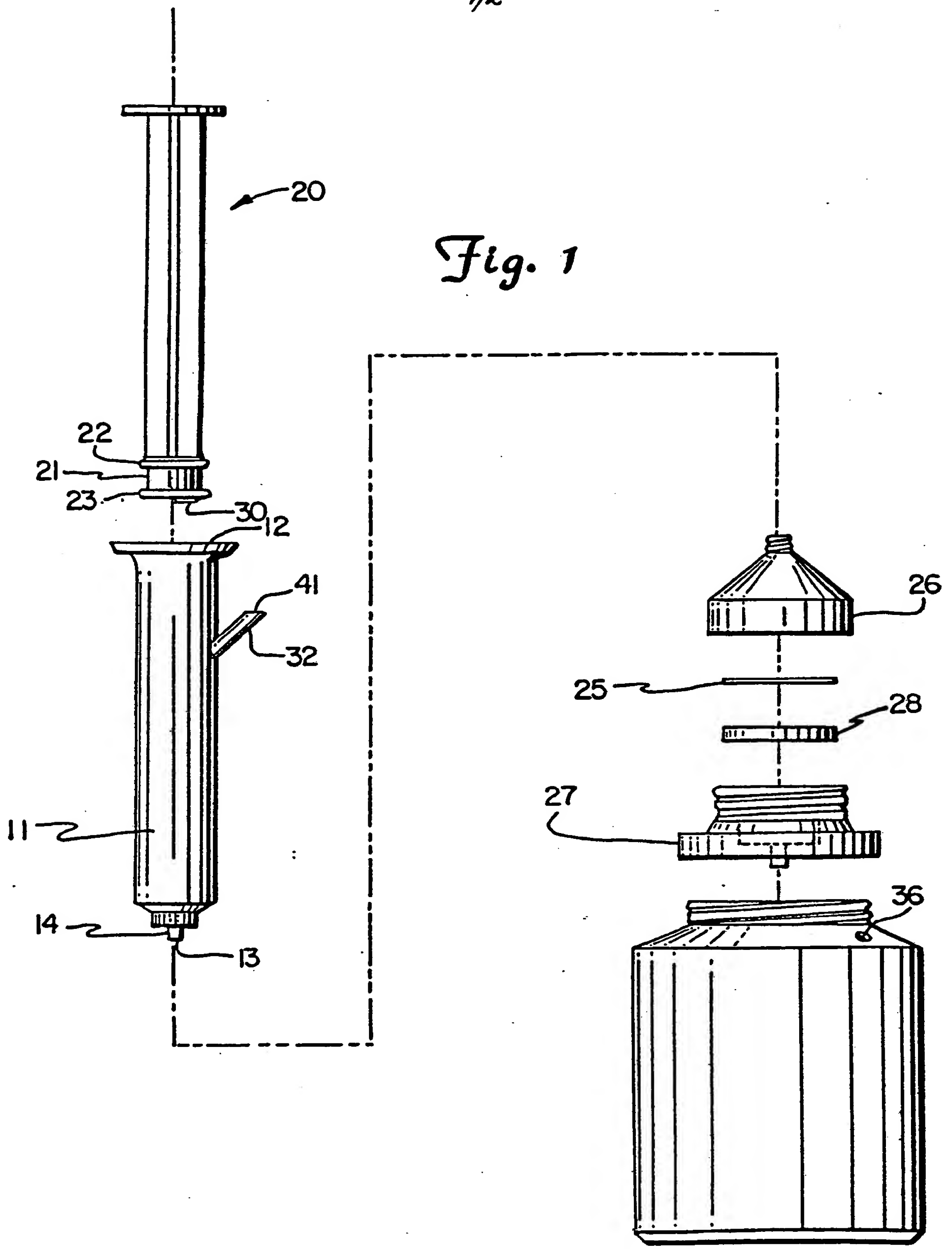

Fig. 1

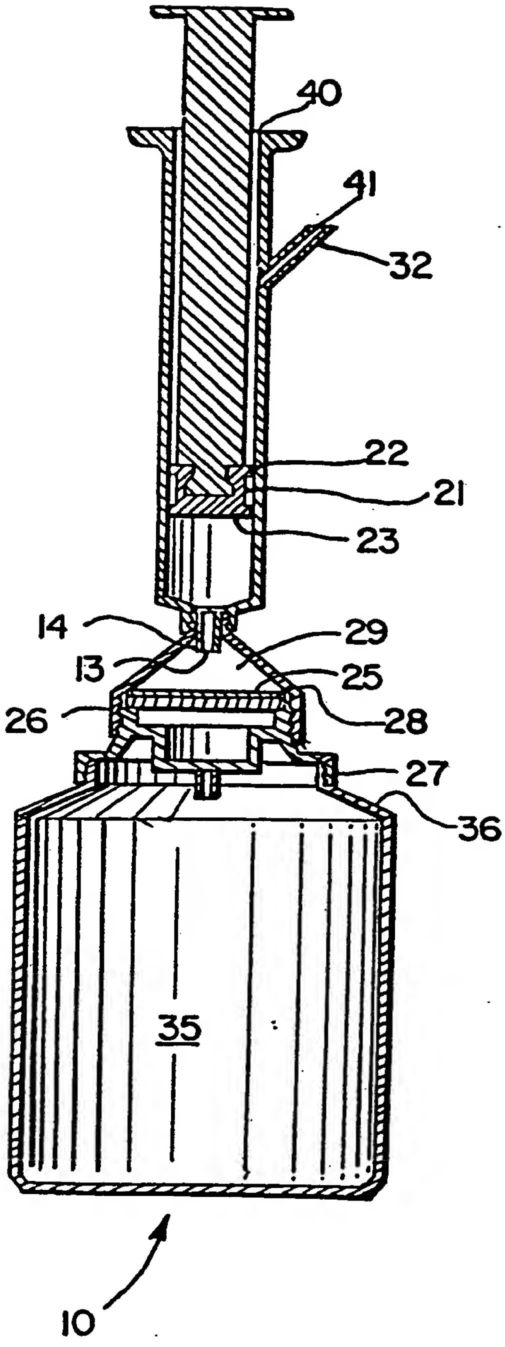

Fig. 2

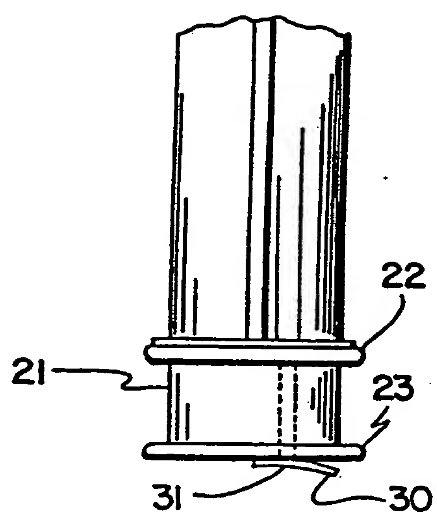

Fig. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/US89/02914

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): G01N 21/78, 33/53, 33/537, 33/538, 33/573
U.S. CL.: 422/58, 61, 101; 435/7; 436/178, 538, 541, 808, 824

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	422/58, 61, 100-102; 435/7; 436/178, 501, 536, 538, 541, 805, 808, 824

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 3,888,629 (BAGSHAWE) 10 June 1975, see the entire document.	1-26
Y	US, A, 4234,317 (LUCAS et al) 18 November 1980, see the entire document.	1-26
Y	US, A, 4,418,702 (BROWN et al) 06 December 1983, see the entire document.	1-26
Y	US, A, 4,734,262 (BAGSHAWE) 29 March 1988, see the entire document.	1-26
Y,P	US, A, 4,775,638 (HAISMA) 04 October 1988, see the entire document.	1-26

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
12 October 1989	09 NOV 1989
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Robert J. Hill Jr. Robert J. Hill, Jr.

Form PCT/ISA/210 (second sheet) (May 1986)